MANGANESE ENHANCED MRI AND LOCALIZATION IN PANCREATIC BETA CELLS

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INTRODUCTION

Due to the ever rising incidence of Diabetes, there is a great need for a non invasive technique able to assess pancreatic β-cell mass and function which would allow for monitoring the disease progression as well as supporting the development of new treatments and therapies. Recently we have shown that Manganese (Mn) enhanced Magnetic Resonance Imaging (MEMRI) can be successfully employed to assess glucose activation and therefore β-cell function in both isolated rodent and human islets (1) as well as in the endogenous pancreas in rodents. Mn has an ionic radius close to that of calcium and is handled in a similar manner in many biological systems. Divalent Mn ions have been shown to enter β-cells through voltage-gated calcium channels (2). Glucose stimulated influx of calcium into ß-cells is necessary for insulin release. When present during glucose stimulation, extracellular Mn can enter ß-cells through voltage-gated calcium channels and its accumulation results in increased MR image contrast. As with rodent islets, high-resolution MRI of glucose activated isolated human islets showed a significant increase in MR contrast. These results were confirmed via X-Ray fluorescence (XFS) on rodent ß-cells which showed intracellular accumulation of exogenous Mn following glucose stimulation.

MATERIALS AND METHODS

Isolated human pancreatic islets were provided by the NIH-ICR.
Static Experiments*:* Islets were incubated in Krebs-Ringer Buffer (KRB) solution with 2mM glucose for 30 minutes. They were then incubated for an additional 30 minutes in either 2mM or 16 mM glucose and 50 mM $MnCl_2$ and then loaded in NMR imaging tubes.
Perfusion Experiments*:* Isolated human islets were loaded in a perfusion chamber and placed in the magnet. Islets were perfused with experimental solutions described above at a rate of 250 ul/ min.
NMR Imaging*:* All experiments were conducted in a 56-mm vertical bore 11.7 T magnet. T1 weighted spin echo images were acquired with TR/TE= 400/7.8 ms, MTX= 128x128. Signal to Noise Ratio (SNR) of unstimulated (2mM glucose) versus stimulated (16 mM glucose) was quantified.
X-Ray Fluorescence: MIN-6 cells were grown 500 nm thick Si_3N_4 windows for 72 hours prior to being exposed to the stimulation paradigm described above and additionally one group was depolarized with 30mM KCl. After the treatment samples were immediately plunge into ethylene cooled with liquid nitrogen. Samples were freeze-dried under vacuum overnight. X-ray fluorescence dataset were acquired on the 2-ID-D beamline at the Advanced Photon Source. Incident X-rays of circa10 keV were generated and focused into a 0.2 × 0.2 μm spot. The sample was raster-scanned through the focal spot with a 1 s. dwell-time. Quantitation and image-processing was performed with MAPS software (3) and standardization.

RESULTS

Consistent with our previous findings in isolated rodent pancreatic islets, Manganese was found to provide an increase in SNR following glucose stimulation (Figure 1). A 10% increase was measured with 50 uM Mn and 30 minutes incubation time in static conditions. Comparable SNR values were obtained with shorter incubation times when islets were perfused. Although MEMRI has found growing applications in recent years for studies of the heart, brain, and pancreatic β-cells, correlation between SNR and intracellular Mn uptake has never been performed at this resolution. Commonly used techniques include Inductively Coupled Plasma Mass Spectrometry which requires a significantly large sample size in the order of thousands of cells. On the contrary, XFS has subcellular resolution and we were able to precisely localized Mn accumulation within the cytoplasm of single β-cells as shown in Figure 2. As expected, untreated samples had only trace concentrations of Mn.

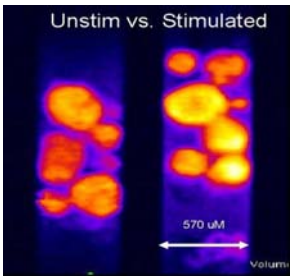

Figure 1. High resolution MR image of isolated human pancreatic islets. Islets were exposed to 50 μM Mn and 2mM (left) and 16 mM (right) glucose respectively.

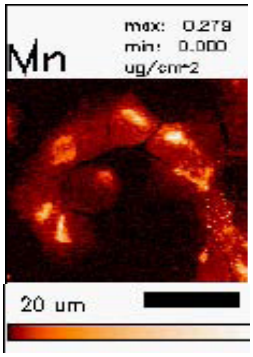

Figure 2. XFS image representing intracellular Mn localization in MIN-6 cells depolarized with 30mM KCl. 0.4 μm in plane resolution.

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